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PROTACs: A Next-Generation Strategy for Targeted Protein Degradation

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Abstract-A new therapeutic approach called proteolysis-targeting chimeras (PROTACs) uses the ubiquitin–proteasome system to specifically break down proteins linked to disease. PROTACs work by bringing a target protein close to an E3 ubiquitin ligase, which causes ubiquitination and subsequent proteasomal destruction, in contrast to traditional small-molecule inhibitors that depend on active site occupancy. This catalytic process increases the number of druggable proteins, eliminates the enzymatic and scaffolding roles of proteins, and produces long-lasting biological effects with lower dosage needs. With uses in immunology, neurological diseases, and oncology, PROTACs have advanced quickly since their invention in 2001. Numerous PROTAC candidates have demonstrated their translational potential by enrolling in clinical studies.

Keywords-PROTAC ,ubiquitin–proteasome system,Proteasome, Proteasomal Degradation, Ubiquitin Recycling.

Introduction-

The ubiquitin–proteasome system (UPS) is used by proteolysis-targeting chimeras (PROTACs), a revolutionary therapeutic approach, to specifically break down disease-associated proteins. In contrast to traditional small-molecule inhibitors, which mainly work by blocking protein activity through occupancy, PROTACs bring a target protein close to an E3 ubiquitin ligase, which catalyzes ubiquitination and the target's eventual proteasomal destruction. Three components make up the structural makeup of PROTACs: a ligand for the target protein, a ligand for an E3 ligase, and a chemical linker that connects the two.Compared to conventional inhibition, this approach has a number of benefits, such as a catalytic mode of action, the capacity to eradicate both the enzymatic and scaffolding activities of proteins, and the potential to overcome resistance brought on by target mutations. Crucially, PROTACs allow for the modification of

proteins that were previously thought to be unmanageable, hence expanding the druggable proteome. PROTACs have advanced quickly since their inception in 2001, with a number of candidates moving forward into clinical trials for inflammatory, neurological, and cancerous conditions. As a result, PROTAC technology offers a revolutionary platform for drug discovery and chemical biology that has potential for next-generation treatments.

Proteosome &Protein Degradation-

The **proteasome** is a large protein complex responsible for degrading unwanted, misfolded, or damaged proteins within the cell. Protein degradation is a highly regulated process in which proteins are broken down into smaller peptides and amino acids. This process is essential for maintaining protein homeostasis, ensuring the removal of defective proteins, and regulating cellular functions. By continuously balancing protein synthesis and degradation, the cell maintains proper function and adapts to various physiological and stress conditions.

How it happens

Ubiquitin-proteosome system(UPS)

Tags proteins for degradation and regulates protein expression level.

Lysosomal proteolysis pathway

Degrades proteins outside of cells including infolded proteins.

Ubiquitin-proteasome pathway-

Within cells, the ubiquitin-proteasome pathway is a tightly controlled mechanism that governs the breakdown of proteins. It eliminates damaged, misfolded, or unnecessary proteins, which is crucial for preserving cellular homeostasis.A little protein known as ubiquitin is used in the route to tag proteins, designating them for proteasome breakdown. Numerous physiological functions, including the cell cycle, signal transduction, gene expression, and stress responses, are crucially regulated by this system.

Ubiquitination: The Tagging Process

A molecule of ubiquitin, a little 76-amino acid protein, is attached to a target protein during the ubiquitination process, designating it for proteasome breakdown.
Ubiquitination happens in a cascade of three steps:

Step 1: Activation (E1) — The ubiquitin-activating enzyme E1 is the first to activate ubiquitin. The ubiquitin molecule and the E1 enzyme create a high-energy thioester bond during activation. ATP is needed for this step.

The second step is conjugation **(E2),** when the ubiquitin-conjugating enzyme (E2) receives the active ubiquitin from E1. Transferring the ubiquitin to the subsequent enzyme in the cascade, E3, is the responsibility of the E2 enzyme.

Step 3: Ligation (E3): The ubiquitin ligase E3 is essential for identifying and attaching to the substrate protein. It facilitates the movement of ubiquitin from E2 to the substrate protein's lysine residue. Only certain proteins are designated for breakdown because E3 ligases are very selective for their target proteins.
Usually joined at lysine 48 of the final ubiquitin molecule in the chain, a polyubiquitin chain—a chain of many ubiquitin molecules—is added to the target protein. The proteasome uses this polyubiquitin chain as a signal to break down the protein.

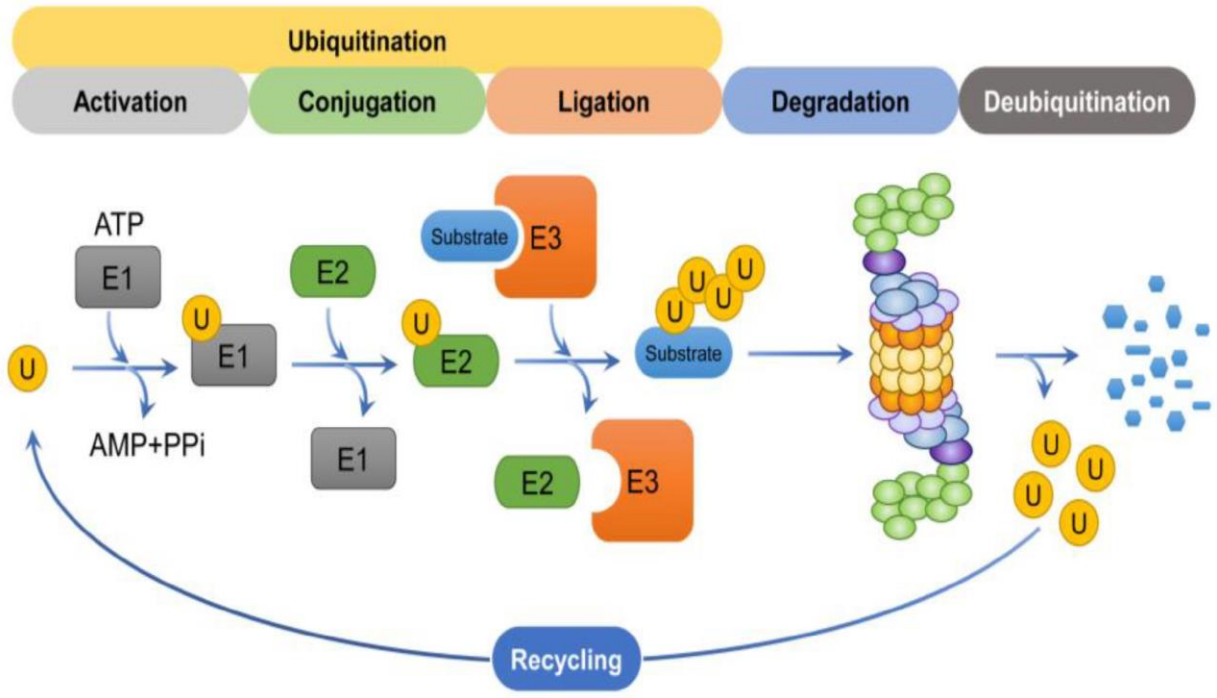

Fig1:Ubiquitination: The Tagging Process

Identifying the Proteasome

The 26S proteasome, which is made up of two primary components, recognizes the target protein after it has been polyubiquitinated.

Protein breakdown takes place in the 20S Catalytic Core Particle (CP), which contains proteases. The substrate protein is broken down into smaller peptides by the protease activity of the 20S core.

Recognizing the polyubiquitin tag and unwinding the target protein are the functions of the 19S Regulatory Particle (RP). Prior to the protein being sent into the catalytic core for breakdown, it also eliminates the ubiquitin chain.

Degradation by the Proteasome

Ubiquitin Removal: The 19S regulatory particle recognizes the polyubiquitin chain on the target protein and removes it. The ubiquitin molecules are recycled and can be reused for subsequent ubiquitination reactions.

Unfolding and Translocation: After the ubiquitin is removed, the target protein is unfolded by the 19S particle, and it is translocated into the 20S core of the proteasome.

Proteolysis: Inside the 20S core, the protein undergoes proteolysis (breakdown) into smaller peptides. The proteasome has several catalytic sites, including **caspase-like**, **trypsin-like**, and **chymotrypsin-like** activities, which cleave the substrate protein at specific sites.

Ubiquitin Recycling

It is possible to tag fresh substrate proteins with the recycled ubiquitin molecules that are extracted from the polyubiquitin chain. Because it enables the effective degradation of several proteins without necessitating the creation of new ubiquitin molecules each time, ubiquitin recycling is an essential component of the process.

PROteolysis Targeting Chimera is referred to as PROTAC. Using tiny compounds to target certain proteins for breakdown instead of just blocking their function is a unique method to drug discovery.

The target protein is drawn to an E3 ubiquitin ligase by PROTACs, which then marks the protein for internal proteasome destruction.

To put it simply, PROTACs work to completely eradicate a protein rather than impede its function, as typical medications do. This may be a more successful way to treat some diseases, particularly cancer and neurological disorders.

Key Components of a PROTAC

The PROTAC's Targeting Ligand (T) is made to attach to the particular protein that you wish to break down. It identifies and attaches itself to the target protein like a "hook" might. Typically, this ligand is a little molecule with a high level of specificity for the target protein.

E3 Ligase Ligand (L): This PROTAC segment attaches itself to an E3 ubiquitin ligase, an enzyme essential for marking proteins for breakdown. The target protein is marked for degradation by the cell's proteasome when ubiquitin molecules are attached to it by the E3 ligase.

Linker: A flexible chemical linker connects the two ligands (the E3 ligase ligand and the targeting ligand). This linker is essential because it enables the two components to align correctly, creating a bridge between the E3 ligase and the target protein.

How PROTAC Works

The PROTAC molecule attaches itself to the particular target protein, such as a protein linked to cancer. The PROTAC is guaranteed to identify and attach to the protein that has to be broken down by the targeted ligand.

E3 Ligase Recruitment: An E3 ubiquitin ligase, which is normally already present in the cell, is bound by the opposite end of the PROTAC molecule. The attachment of ubiquitin molecules to the target protein is catalyzed by the enzyme E3 ligase.

Bringing the Target and E3 Ligase Together: The PROTAC molecule's flexible linker enables it to bring the target protein and E3 ligase together to form a stable complex.

Ubiquitination: The target protein now has a chain of ubiquitin molecules added by the E3 ligase. A little protein called ubiquitin marks other proteins for breakdown by the proteasome, a big complex in cells that eliminates undesirable or damaged proteins.

Proteasomal Degradation: The proteasome recognizes the target protein once it has been ubiquitinated, unfolds it, and translocates it into the proteasomal core. Inside the proteasome, the protein is digested into small peptides, ultimately eliminating it from the cell.

Continued Targeting: PROTACs can provide longer-lasting inhibition because they cause the target protein to continuously degrade rather than only stop its activity. Additionally, the PROTAC is reusable and not consumed during the degradation process, in contrast to conventional inhibitors.

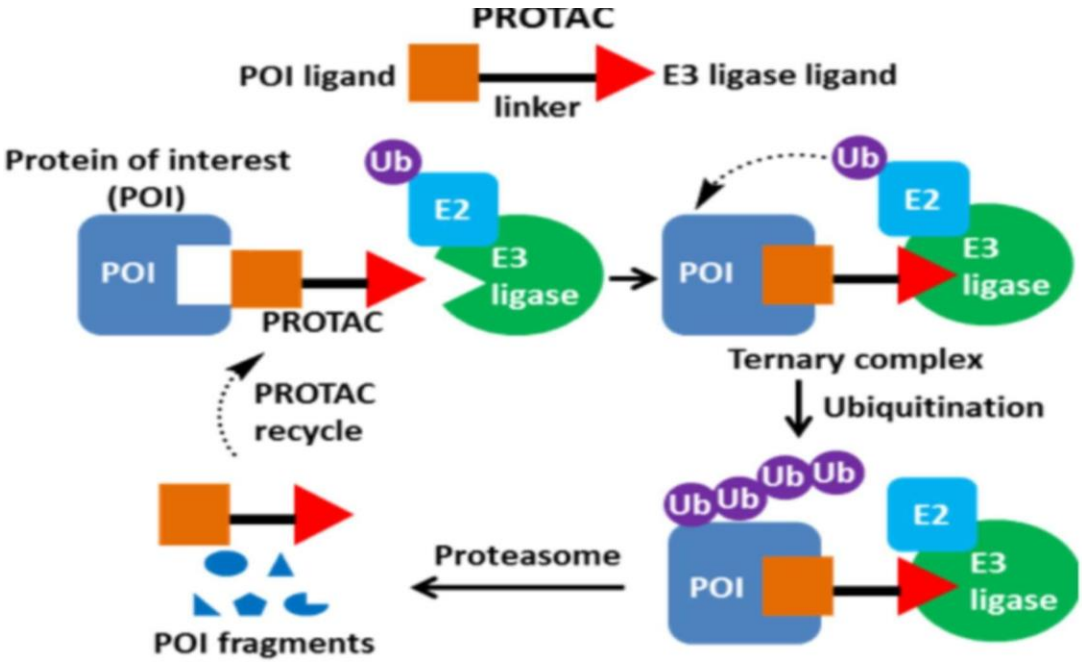

Fig-2 how protac works

Example: PROTAC Targeting Cancer Proteins

PROTACs can be engineered to break down particular proteins, like oncogenes or survival factors, that are overexpressed or mutated in cancer. For instance, an E3 ligase may be recruited by a PROTAC to tag a mutant BCL-2 protein, which is important in blocking cancer cell death, for destruction.

The cancer cell would lose a crucial survival component as a result, which could result in cell death or slower tumor growth.

Advantages of PROTACs:

Selective degradation: PROTACs can degrade specific proteins with high selectivity, even those that can't be inhibited with traditional small molecules.

Overcoming resistance: Because PROTACs degrade proteins rather than inhibiting them, they may overcome resistance mechanisms that arise from mutations in target proteins.

Continuous activity: PROTACs degrade the target protein continuously, potentially providing more sustained effects compared to traditional drugs.

Diseases Associated with Ubiquitin-Proteasome Dysfunction

Neurodegenerative Diseases: Impaired protein degradation due to dysfunction in the ubiquitin-proteasome system (UPS) leads to the accumulation of misfolded proteins, a hallmark of

conditions like Alzheimer's, Parkinson's, and Huntington's diseases.

Cancer: Dysregulated protein degradation can lead to the inappropriate accumulation of oncogenic proteins (e.g., **myc**, **Cyclin D1**) or the failure to degrade tumor suppressor proteins, contributing to cancer progression.

Cardiovascular Disease: Protein misfolding and malfunction in the heart can lead to heart failure, where the UPS is involved in regulating proteins essential for cardiac function.

Conclusion

With clear benefits over conventional small-molecule inhibitors, PROTACs have become a potent and adaptable strategy for targeted protein breakdown. PROTACs provide long-lasting and catalytic therapeutic effects by using the ubiquitin–proteasome system to destroy disease-associated proteins instead of just blocking their activity. The druggable proteome is increased, previously unreachable targets can be modulated, and resistance mechanisms may be circumvented with this approach. Even if there are still issues with pharmacokinetics, selectivity, and possible off-target effects, PROTAC technology has the potential to revolutionize medicine, as demonstrated by continuing research and clinical studies. PROTACs are anticipated to be crucial in the creation of next-generation treatments in neurodegeneration, oncology, and other areas as the field develops.

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